Results: Of the 351 subjects enrolled in the MeTeOR Trial, 292 had complete data on outcomes and covariates used in this analysis. 57% were female and mean age was 58 (SD 7). 141 of these subjects were randomized to APM and received surgery. Another 93 subjects were randomized to PT and continued to receive PT, while 58 were randomized to PT and crossed over to receive APM. Forty percent of cross-overs occurred within three months of randomization. In multivariable analyses, factors associated with a higher likelihood of crossing over to have APM among those randomized to PT included baseline WOMAC Pain score > 40 (RR 1.13, 95% CI: 1.05, 1.20), duration of symptoms less than one year (RR 1.08, 95% CI: 1.01, 1.15) and ability to extend the knee fully (RR 1.07, 95 % CI: 0.99, 1.16). The model had a c-statistic of 0.70. The proportion of subjects who achieved a 10 point improvement in pain score after six months of follow-up ranged from 80% in those randomized to APM, 73% in those randomized to PT who did not cross over and 69% in subjects who crossed over to APM. In multivariate analyses, those who crossed over to APM were 23% less likely to achieve a 10-point improvement in pain score than subjects originally randomized to APM (RR 0.77 95% CI: 0.54, 0.92), adjusting for baseline pain and duration of symptoms.
Conclusions: These data suggest that patients assigned to PT who were most likely to cross over to APM included those with a more acute and painful onset - characterized by short duration of symptoms, high level of pain and least extension loss. The findings also suggest that patients who cross over to surgery are 23% less likely to experience improvement in pain than those originally randomized to APM. This difference should be interpreted with care as the groups are not randomized. Taken together, these findings suggest a need for 1) further research to identify patients for whom initial PT is less likely to be successful and 2) strategies to support patients who do not improve with PT, as the results of APM in these patients may be worse than in those treated with APM at the outset.

22
THE ROLE OF ERYTHROMYCIN/GHRELIN AXIS IN ARTICULAR CARTILAGE MAINTENANCE

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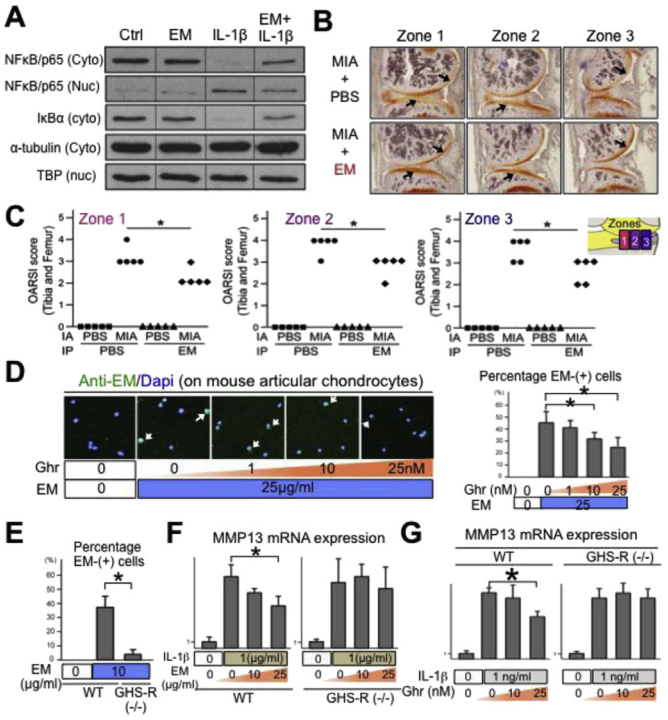
Purpose: The goal of this research was to investigate the effects of a well-known antibiotic, erythromycin, and a well-known "hunger hormone", ghrelin, on chondrocytes. In recent years, certain macrolide antibiotics were found to combat inflammation in the lung and the bone, even though they did not affect bacterial growth at the concentrations used, suggesting that this activity is independent of its antibiotic properties. Among the macrolide antibiotics, only EM has been tested in one human OA clinical trial, which showed reduction of joint effusion and pain over the course of 4 months, but how EM achieved such an effect was not assessed in this study.
Ghrelin is a 28 amino acid long peptide best known as the "hunger hormone", since its level is highest before meals and drops after meals. Ghrelin is reported to have protective effects against pulmonary and brain injuries in combating inflammation and pain. As labeled EM has been reported to bind to purified ghrelin receptor, we investigated whether EM acts through ghrelin receptor on chondrocytes, and whether ghrelin has a similar activity on chondrocyte gene expression.
Methods: In vitro: Normal human articular chondrocytes (Lonza) or mouse immature articular chondrocytes were cultured in the presence or absence of IL-1β, erythromycin and ghrelin (varying amounts) for 4 days followed by RT-PCR. For Western blot analysis of NFkB signaling, samples were harvested 1hr after IL-1β treatment. In vivo: CD1 mice were injected either with monosodium iodoacetate (MIA, 62.5μg) intrarticularly or erythromycin (400 μg) or both, with PBS controls (6/group). Immunohistochemistry (IHC) was performed 10 days later, and cartilage damage was quantified by the OARSI scoring system. Ex vivo: human OA cartilage slices were cultured in the presence of ghrelin or PBS for 3 weeks and matrix level was analyzed by IHC and safranin/O staining. Data are reported as mean ± standard deviation. For parametric data, statistical analysis was performed using a one-way analysis of variance followed by post-hoc Tukey test. For nonparametric data (OARSI scores), statistical analysis was performed using the Kruskal-Wallis test followed by Mann-Whitney U test with Bonferroni correction. P-values of <0.05 were considered significant.



Results: 1. Erythromycin inhibited IL-1β induced collagen II reduction and iNOS, MMP1, 3, 9, 13 induction in human chondroctyes (data not shown), and inhibited IL-1β-induced NFκB nuclear localization (Fig. 1A). To evaluate the effect of erythromycin on cartilage damage in vivo, we injected MIA into the knee joint. This model is an OA and pain model because it demonstrates all features of injury-induced OA, even though MIA is not present naturally. We found that erythromycin does not affect cartilage matrix production in normal conditions, but significantly reduced MIA-induced matrix loss, even though different medial-lateral zones have different extent of damage (Fig. 1B and 1C).
2. By utilizing an anti-erythromycin antibody, we found dosage-dependent binding of erythromycin to mouse articular chondrocytes after just 3hr or 1 day of erythromycin addition (Fig. 1D). Consistent with a prior report of erythromycin binding to the ghrelin receptor GHS-R1, increasing levels of ghrelin administration displaced erythromycin binding to the chondrocytes (data not shown). Furthermore, such binding was diminished in chondrocytes from the GHS-R KO mice (Fig. 1E). Correspondingly, erythromycin did not inhibit MMP13 expression in GHS-R KO chondrocytes (Fig. 1F).
3. Ghrelin administration inhibited IL-1β-induced iNOS and MMP expression in human chondrocytes (data not shown), and this activity was lost in chondrocytes from GHS-R KO mice (Fig. 1G). Furthermore, ghrelin inhibited IL-1β-induced NFκB nuclear localization in chondrocytes, and increased cartilage matrix levels in human OA cartilage explant cultures (data not shown).
Conclusions: This work provides a novel link of two molecules that are not known to regulate chondrocyte gene expression. We show that erythromycin and ghrelin act through the ghrelin receptor to inhibit catabolic activities in articular chondrocytes, which may represent a new mechanism in the control of matrix loss in arthritis.

23
DIO2-DEFICIENT MICE ARE PROTECTED AGAINST CARTILAGE DAMAGE IN A MODEL OF EXERCISE-INDUCED OA

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Purpose: Previously, we identified the DIO2 gene as human OA susceptibility gene and showed detrimental DIO2 up-regulation in OA affected cartilage. Additionally, we showed that pharmacological inhibition of DIO2 signaling in a human 3D chondrogenesis cell-model confers prolonged cartilage matrix homeostasis. Here we aim to investigate the beneficial effect of deficient deiodinase iodothyronine type-2 (DIO2) in OA by studying the effects of forced mechanical loading on in vivo joint cartilage tissue homeostasis in Dio2 knockout mice.
Methods: Wild-type and C57BL/6-Dio2 -/- -mice were subjected to a stringent, forced running regime for one hour per day for three weeks. Severity of osteoarthritis was assessed by histological scoring for cartilage damage and synovitis. Genome wide gene expression was determined in knee-cartilage by microarray analysis (Illumina MouseWG-6 v2). STRING-db analyses were applied to determine enrichment for specific pathways and to visualize protein-protein interaction (PPI)-networks.
Results: Dio2-deficient mice showed significant less cartilage damage ($P < 0.0001$) and reduced severity of synovitis ($P = 0.0184$) after administration to a treadmill running model of OA. In the wild-type-specific and the overall analyses we observed in total, 158 probes representing 147 unique genes significantly differential expressed with a fold-change $\geq$ 1.5 upon forced exercise. Among these are genes known for their association with OA (e.g. Mef2c, Egfr, Ctgf, Prg4 and Ctnnb1), supporting the use of forced running as an OA-model in mice. However, in the knockout-specific analysis, we observed no significant differential expression in articular cartilage upon the running regime, suggesting an attenuated response to biomechanical burden. Among the differential expressed genes, thyroid signaling was found to interact directly with the differential expressed genes through, Ctgf and Egfr, via thyroid receptor alpha (Thra) and retinoid x receptor (Rxra). Dio2-deficient mice showed significantly less cartilage damage and signs of synovitis.
Conclusions: We find that Dio2 deficiency has a protective effect on the homeostasis of articular cartilage in the knee-joints of mice undergoing a forced running regime. This is consistent with our earlier findings, showing that pharmacological inhibition of deiodinases in a human in vitro chondrogenesis model has a beneficial effect on the early formation and maintenance of articular cartilage ECM. It is therefore hypothesized that control of thyroid hormone signaling, both during development and adult cartilage maintenance, is essential to ensure normal bone and cartilage homeostasis, and that it could act as the master-switch that forces maturational arrested chondrocytes to re-activate the endochondral ossification process, leading to articular cartilage destruction. Our results show that interfering with intracellular thyroid hormone levels could be a powerful way to oppose the pathological events that are occurring in OA.

24
SYNOVIAL MACROPHAGES PROMOTE TGF-β SIGNALING AFTER INTRA-ARTICULAR INJECTIONS OF OXIDIZED LDL IN NAÏVE MURINE KNEE JOINTS, PREVENTING PRODUCTION OF PRO-INFLAMMATORY FACTORS S100A8/9, CHEMOKINES AND AGGRECANASE-INDUCED NEO-EPITOPES

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Purpose: In previous studies we found that synovial macrophages regulate joint pathology during experimental osteoarthritis (OA) and, more recently, we found that high systemic levels of LDL aggravate joint pathology during experimental OA with synovitis. LDL in inflamed synovium is oxidized and taken-up by macrophages via scavenger receptor A and CD36, leading to an activated macrophage phenotype. In this study, we investigate whether direct injection of oxLDL into a normal murine knee joint induces joint pathology and elucidate the role of synovial macrophages in that process.
Methods: Knee joints of C57BL/6 mice were injected at five consecutive days with 6 μL (1.2 mg/mL) oxLDL, LDL, or an equal volume of vehicle (PBS). This same procedure was done in mice which were depleted of synovial macrophages by intra-articular injection of clodronate liposomes seven days prior to the (ox)LDL or vehicle injections. Joint pathology was investigated by immunohistochemistry and RNA expression and protein production by synovium were determined using RT-PCR and luminex, respectively. Active TGF-β was measured using a functional CAGA-luciferase assay. Data are depicted as mean ± standard deviation.
Results: LDL and oxLDL injection in naïve knee joints did not increase synovial thickening, or production of pro-inflammatory factors (IL-1β, IL-6 and S100A8/9) compared to vehicle injection. Levels of active TGF-β in synovial wash-outs was, however, significantly increased by 33% (from 84.7 ng/mL/g synovium ± 14.4 to 113.0 ng/mL/g synovium ± 33.3; $p<0.05$). Immunohistochemistry of total knee joints showed that oxLDL injection decreased formation of aggrecanase-induced neo-epitopes (NITEGE) compared with vehicle injections (3.6 times; $p<0.05$), especially in areas along the bone margins that are prone to develop osteophytes (from arbitrary score 1.19 ± 0.57 to 0.33 ± 0.30; $p<0.05$). In contrast, repeated injections of oxLDL in macrophage-depleted knee joints led to a 3.1 fold increase of synovial thickening (due to cell influx), compared with injection of vehicle ($p<0.01$), while LDL injections did not alter synovial thickening. Protein levels of S100A8/A9, markers for inflammation, were significantly increased in synovial wash-outs of oxLDL injected joints, compared with LDL (fold increase 5.6; $p<0.05$) or vehicle (fold increase 8.3; $p<0.01$) injection. RNA levels of chemokines CCL2 (Mcp-1) and CCL3 (Mip-1α) were also significantly upregulated after oxLDL injections (6.7 fold and 4.6 fold, respectively; $p<0.01$). No raise in active TGF-β was measured in macrophage-depleted joints. NITEGE expression was markedly increased (fold increase 1.92) in the synovial-cartilage contact areas after oxLDL injection ($p<0.05$).
Conclusions: Synovial macrophages promote anabolic effects after oxLDL injections in knee joints, supporting earlier studies which show increased ectopic bone formation during LDL-rich conditions in experimental osteoarthritis. In absence of synovial macrophages, however, oxLDL induces cell influx, production of pro-inflammatory mediators and aggrecanase activity, thereby increasing catabolic activity.

25
CONDITIONAL DELETION OF CTGF REVEALS A PIVOTAL ROLE FOR CTGF ON ACTIVATING THE LATENT COMPLEX OF TGFβ

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Purpose: Work from our lab has identified an important role for the pericellular matrix (PCM) of cartilage sequestering regulatory molecules such as FGF2, latent TGFβ and connective tissue growth factor (CTGF). In rested cartilage these molecules are held back from the cell surface, but upon mechanical stimulation they are released causing cellular activation (Vincent et al. 2007, and unpublished results). Data presented at OARSI 2014 revealed that CTGF and latent TGFβ are released in a high molecular weight complex upon cartilage injury. In this study we extend these results to address how CTGF controls the release and activation of latent TGFβ.
Methods: CTGF floxed mice were crossed with ubiquitin Cre-ERT2 mice to generate post natal, pan-tissue, tamoxifen-inducible CTGF knockout mice. Femoral head avulsion of wild type or knockout tissue into serum free medium was used as an injury model. Medium conditioned for up to 24 hours was analysed for CTGF and TGFβ protein, and phosphoSMAD2/3 activity. Selected cell surface receptors were blocked to assess their involvement in activation of latent TGFβ in the cut conditioned medium.
Results: Medium conditioned by avulsion injury of CTGF knockout murine cartilage confirmed reduced levels of CTGF protein. Surprisingly, levels of TGFβ in the injury CM was similar between wild type and knockout tissues suggesting that CTGF was not required for the release of latent TGFβ from the pericellular matrix. However, injury CM generated from CTGF knockout cartilage showed reduced SMAD2/3 activity in isolated porcine chondrocytes compared with activity from injured wild type cartilage, indicating that CTGF is involved in the activation of latent TGFβ at the cell surface. Incubation of porcine chondrocytes with either an RGD- integrin binding peptide or a blocker of LRP1 (scavenger receptor which is known to internalise CTGF) did not affect SMAD2/3 activity in the injury medium suggesting that these known binding partners of CTGF are unlikely to be involved in the CTGF-dependent activation of latent TGFβ.
Conclusions: Our results show CTGF has a critical role in activating latent TGFβ at the cell surface and is not required for the release of latent TGFβ from the pericellular matrix of cartilage upon injury.

26
PHLPP1 DELETION INCREASES FGF18 EXPRESSION AND PROTECTS AGAINST SURGICALLY-INDUCED OSTEOARTHRITIS

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